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TECHNICAL MANUSCRIPT 451

NEUTRALIZATION OF SHELLFISH POISON
BY CHEMICAL DISINFECTANTS

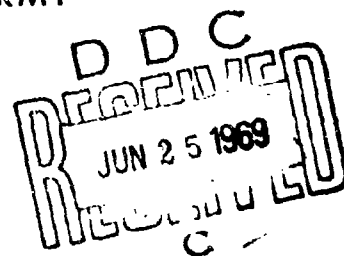
Choo D. Chin

MAY 1969

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DEPARTMENT OF THE ARMY
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TECHNICAL MANUSCRIPT 451

NEUTRALIZATION OF SHELLFISH POISON BY CHEMICAL DISINFECTANTS

Choo D. Chin

Research & Radiological Division
INDUSTRIAL HEALTH & SAFETY DIRECTORATE

Project 1B662706A072

May 1969

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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ABSTRACT

The neutralization of shellfish poison by 11 chemical disinfectants was studied. Both sodium hypochlorite and calcium hypochlorite were effective in neutralizing the toxicity of the poison at 3 ppm/ μ g of poison at room temperature (25 C) and at 4 ppm/ μ g of poison at 4 C. Alkali in the hypochlorite solutions tends to enhance the effectiveness of these disinfectants.

Potassium permanganate was effective as a poison neutralizer at 50 ppm/ μ g of poison at 25 C.

Sodium hydroxide, ethylene oxide, beta-propiolactone, Lysol, formaldehyde, iodine, peracetic acid, and Roccal were not as effective as the hypochlorites and the permanganate.

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I. INTRODUCTION*

Paralytic shellfish poison has long been known to be toxic for man. Ingestion of as little as 1 mg of the purified poison may be fatal.^{1,2} Because there is no known antidote, working with the concentrated material is not altogether without hazard.

Heating the poison in an autoclave at 20 psig (121 C) for 24 hours has no effect on its toxicity. The neutralizing effect of chemicals has not been thoroughly studied, although Brazis et al.³ evaluated the stability of the poison in the presence of chlorine dioxide and free available chlorine. They found that more than 93% of the poison (4.7 mg/liter) was destroyed within 2 hours, but free available chlorine had no effect.

The present study is an attempt to find a practical decontaminating agent for use in the laboratory that will neutralize the poison upon contact. Eleven common chemical disinfectants were tested.

II. MATERIALS AND METHODS

A. TEST CHEMICALS

- 1) Sodium hydroxide (NaOH) at 0.01, 0.05, and 0.3%
- 2) Sodium hypochlorite (NaOCl) at various concentrations
- 3) Calcium hypochlorite [$\text{Ca}(\text{OCl})_2$] at various concentrations
- 4) Iodine (I_2) at 0.5% in 1.5% KI solution
- 5) Formaldehyde (HCHO) at 0.5%
- 6) Beta-propiolactone (BPL) at 1%
- 7) Roccal at 1%
- 8) Lysol at 5%
- 9) Peracetic acid at 1%
- 10) Potassium permanganate (KMnO_4) at 0.01, 0.05, and 0.1%
- 11) Ethylene oxide gas at 12 g/ft³

B. SHELLFISH POISON

Unless otherwise mentioned, the reference standard purified shellfish poison at 100 µg/ml in 0.1 N HCl containing 20% ethanol was used throughout the tests. Distilled water was used as the poison diluent. The poison was originally isolated from Alaska butter clams by Dr. Schantz.

* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the author to ascertain when and where it may appear in citable form.

C. EXPERIMENTAL PROCEDURES

The method for testing each disinfectant against the shellfish poison was essentially the same. A known amount of disinfectant at a known concentration was allowed to react with a known amount of poison for a certain time. In discussing results, this mixture hereafter is referred to as the "test solution(s)." The ratio of disinfectant to shellfish poison was either 9:1 or 1:1 by volume, and the exposure time varied from 1 minute to 16 hours, depending on the chemical being tested. At the end of the exposure period, the disinfectant was neutralized if possible. Then the final solution was adjusted to pH 3.0 with the 1.0 or 5.0 N HCl, and 0.4 ml of this acidified solution was inoculated intraperitoneally into mice (18 to 22 g). Because one mouse unit* is equal to 0.183 μ g of purified shellfish poison, with a ratio of disinfectant to shellfish poison of 9:1, an inoculum of 0.4 ml contained 4 μ g of poison or 22 mouse units. At a 1:1 ratio, 0.4 ml of the inoculum contained 20 μ g of poison or 109 mouse units. Reduction of toxicity of the poison was based on the number of mouse units present in the inoculum. Four mice were inoculated per test. Survival of the inoculated mice was considered an indication that the poison was inactivated. Details of the tests with each disinfectant are described separately. Unless otherwise mentioned, all tests were conducted at room temperature (25 C).

III. EXPERIMENTS WITH TEST CHEMICALS

A. SODIUM HYDROXIDE

1. Test Procedure

Into a 100-ml beaker containing 9 ml of the 0.01% NaOH solution, 1.0 ml of the diluted (10 μ g/ml) shellfish poison was added and mixed thoroughly. The mixture was allowed to react for 1, 4, or 24 hours. Then 2-ml aliquots of the mixture were pipetted out and neutralized dropwise with 5 N HCl to pH 3.0. The acidified solution was inoculated into the mice.

A volume of 10 ml of the 0.01% NaOH solution acidified to pH 3.0 was used as the NaOH controls. For the poison controls, a 1:99 dilution of the poison was used.

Similar tests were conducted with the 0.05 and 0.3% NaOH solutions.

* A mouse unit is defined as the amount of poison that will kill a 20-g mouse in 15 minutes.

2. Results

Results are given in Table 1. From the median death time of the mice, the number of mouse units contained in the inoculum was determined from Sommer's tables. These tables are based on graphs recorded in the literature⁴ and are also found in the interim plan issued by the Public Health Service.⁵ The tables provide the death-time:mouse-unit relationships for paralytic shellfish poison. In terms of mouse units, it was calculated that approximately 53% of the poison was destroyed by the 0.3% NaOH solution in 24 hours. The results tend to confirm the finding by Schantz et al.,⁶ who noticed a slow decomposition of the poison when purification was carried out in alkaline solutions, especially at pH values greater than 8 or 9.

TABLE 1. EFFECT OF NaOH ON SHELLFISH POISON^{a/}

NaOH, %	Approx. pH of Mixture	Contact Time, hours	Mortality of Mice ^{b/}	Poison Concn ^{c/}	Inactivation, %
0.01	9.2	1	4/4 (4:18)	5.80	2
0.01	9.2	4	4/4 (4:40)	5.20	12
0.01	9.2	24	4/4 (4:20)	5.65	4
0.05	11.5	1	4/4 (4:42)	5.02	15
0.05	11.5	4	4/4 (4:40)	5.20	12
0.05	11.5	24	4/4 (4:40)	5.20	12
0.3	12.3	1	4/4 (4:08)	5.80	2
0.3	12.3	4	4/4 (4:21)	5.65	4
0.3	12.3	24	4/4 (10:15)	2.75	53
0.01 Control			0/4		
0.05 Control			0/4		
0.3 Control			0/4		
Shellfish poison control (1:99 dilution)			4/4 (4:12)		

- Concentration of shellfish poison in test solution was calculated to be 5.90 mouse units/ml prior to the test.
- Number dead/number tested (average minutes:seconds to death).
- Concentration of shellfish poison in test solution after test, mouse unit/ml.

In one of their studies on the chemical properties of the shellfish poison, Schantz and his co-workers⁷ also noticed that the poison was slowly oxidized by exposure to air in alkaline solution. By ultraviolet absorption, these investigators followed the reaction to its completion. They found that, with 1 N (4%) NaOH solution, 4 days were required for the reaction to be completed when 0.5 ml of the poison (4.31 mg/ml) was mixed with 9.5 ml of the alkaline solution at room temperature. Six days were needed if 0.5 N (2%) or 0.25 N (1%) were used.

B. SODIUM HYPOCHLORITE

The NaOCl used in these tests* contains 10% NaOCl by weight. Chemical analysis indicated that it contained 10% available chlorine at the time of the test. The hypochlorite was stored at 4 C to retard decomposition. A fresh solution was prepared for each test. Freshly prepared solutions of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) at various concentrations were used as the NaOCl neutralizer.

1. Experiment 1 with Sodium Hypochlorite

a. Test Procedure

One milliliter of the shellfish poison was added to 9 ml of 0.5% NaOCl solution in a test tube. The solutions were mixed thoroughly. After 15, 30, and 60 minutes, 2-ml aliquots of the mixture were removed and neutralized with 0.2 ml of 10% $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ solution. The neutralized solution was acidified to pH 3.0 with 1 N HCl before injecting into the mice.

Two milliliters of the 0.5% NaOCl solution neutralized with 0.2 ml of the 10% $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ were used as the NaOCl control. Two milliliters of the 1:9 dilution of the shellfish poison treated similarly with the 10% $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ were used as the poison control.

b. Results

None of the mice died from either the test solutions or from the NaOCl controls. All the mice from the poison-control group died, and the median death time of 1 minute and 40 seconds indicated a 55% loss of toxicity of the poison. This loss is probably due to the salt effect of the $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ solution. Schantz et al.,⁸ who studied this salt effect, found that the presence of NaCl in the reference standard at 0.5 and 1.0% lowered the recovery of the poison to 69 and 55%, respectively. Wiberg and Stephenson,⁹ in their toxicologic studies on paralytic shellfish poison, found that the toxicity of the poison in mice is lowered by the presence of NaCl in the injection medium.

2. Experiment 2 with Sodium Hypochlorite

a. Test Procedure

The results of the first experiment indicated that a rapid and complete neutralization might be obtained with a higher relative concentration of NaOCl. To determine the optimum mixture, a series of tests was conducted using concentrations of NaOCl solutions ranging from 0.001 to 0.05%. Two sets of tests were conducted: one set with a ratio of NaOCl to shellfish poison of 9:1 (v/v) and another with a ratio of NaOCl to shellfish poison of 1:1 (v/v). In the latter set of tests, 5 ml of the

* Manufactured by Du-Rite Chemical Co., 3800 37th Place, Brentwood, Maryland 20722.

poison and 3 ml of the desired NaOCl solution were used per test. The contact times in these tests were 1, 15, and 30 minutes. The amounts and concentrations of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ solution used to neutralize the various concentrations of NaOCl solutions were adjusted according to the concentration of the NaOCl in each test. Solutions of NaOCl at 0.005 and 0.05% neutralized with the $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ solutions were used as the NaOCl controls. Shellfish poison diluted with distilled water at 9:1 and 1:1 dilutions treated similarly with the thiosulfate solutions were used as the poison control.

b. Results

The results of these two sets of tests (Table 2) show that NaOCl at concentrations of 0.003 and 0.03% inactivated 10 and 100 μg of shellfish poison, respectively. None of the concentrations of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ used in these tests affected the toxicity of the shellfish poison.

3. Experiment 3 with Sodium Hypochlorite

a. Test Procedure

To trace the end point of inactivation a little further, another set of tests was conducted using solutions of NaOCl between 0.025 and 0.03%. The ratio of NaOCl to shellfish poison was 1:1 (v/v) and the contact time 15 minutes. Only one contact time was studied in order to conserve the poison supply.

b. Results

Results appear in Table 3. Complete inactivation of the poison was not achieved in 15 minutes when the concentration of NaOCl was below 0.03%. However, in one test in which the contact time was increased to 30 minutes with the 0.029% NaOCl solution, none of the inoculated mice died, but the mice remained ill for 4 hours immediately after the inoculation. These tests indicated that the threshold for complete inactivation lies between the 0.029 and the 0.03% NaOCl solutions.

4. Experiment 4 with Sodium Hypochlorite

a. Test Procedure

On the basis of preceding results, it appeared that the minimum concentration of NaOCl needed for complete inactivation under the above test conditions was 3 ppm/ μg of poison. To prove the validity of these findings, another set of tests was conducted based on the 3:1 ratio (3 ppm/ μg). Solutions of shellfish poison and of NaOCl were prepared in various concentrations so that, when the two solutions were mixed together at a ratio of 1:1 (v/v), they contained 3 ppm of NaOCl/ μg of shellfish poison.

TABLE 2. EFFECT OF NaOCl ON SHELLFISH POISON

Ratio of NaOCl to Poison, $\frac{a}{b}$ 9:1 (v/v)					Ratio of NaOCl to Poison, $\frac{b}{c}$ 1:1 (v/v)						
NaOCl, %	Approx. pH of Mixture	Contact Time, minutes	Mortality of Mice $\frac{d}{e}$	Poison Conc'd/ Conc'd	Inact., %	NaOCl, %	Approx. pH of Mixture	Contact Time, minutes	Mortality of Mice $\frac{f}{g}$	Poison Conc'd/ Conc'd	Inact., %
0.005	4.5	1	2/4 (8:00)	3.1	95	0.05	5.6	1	0/4	0	100
0.005	4.5	15	0/4	0	100	0.05	5.6	15	0/4	0	100
0.005	4.5	30	0/4	0	100	0.05	5.6	30	0/4	0	100
0.004	4.5	1	4/4 (4:26)	5.5	92	0.04	5.5	1	0/4	0	100
0.004	4.5	15	0/4	0	100	0.04	5.5	15	0/4	0	100
0.004	4.5	30	0/4	0	100	0.04	5.5	30	0/4	0	100
0.003	4.5	1	4/4 (3:15)	8.3	87	0.03	5.0	1	4/4 (2:06)	17.6	92
0.003	4.5	15	0/4	0	100	0.03	5.0	15	0/4	0	100
0.003	4.5	30	0/4	0	100	0.03	5.0	30	0/4	0	100
0.002	4.0	1	4/4 (1:36)	3.5	47	0.02	4.5	1	4/4 (1:59)	19.2	93
0.002	4.0	15	4/4 (3:40)	7.0	89	0.02	4.5	15	4/4 (2:04)	17.6	92
0.002	4.0	30	2/4 (6:56)	3.5	95	0.02	4.5	30	0/4	0	100
0.001	3.5	1	4/4 (1:22)	62.0	6	0.01	3.5	1	4/4 (1:04)	250	8
0.001	3.5	15	4/4 (1:25)	52.0	20	0.01	3.5	15	4/4 (1:17)	96	65
0.001	3.5	30	4/4 (1:30)	41.0	38	0.01	3.5	30	4/4 (1:35)	250	8
0.005 Control			0/4			0.05 Control			0/4		
Shellfish poison control			4/4 (1:20)			Shellfish poison control			4/4 (1:00)		
(1.9 dilution)						(1:1 dilution)					

a. Concentration of shellfish poison in test solution was calculated to be 66 mouse units/ml prior to the test.

b. Concentration of shellfish poison in test solution was calculated to be 273 mouse units/ml prior to the test.

c. Number dead/number tested (average minutes:seconds to death).

d. Concentration of shellfish poison in test solution after the test, mouse unit/ml.

TABLE 3. INACTIVATION OF SHELLFISH POISON WITH NaOCl;
 RATIO OF NaOCl TO SHELLFISH POISON^a 1:1 (v/v);
 CONTACT TIME, 15 MINUTES

NaOCl, %	Approx. pH of Mixture	Mortality of Mice ^b	Poison Concn ^c	Inactivation, %
0.025	4.5	4/4 (1:00)	273	0
0.026	4.5	4/4 (1:45)	26	90
0.027	4.5	4/4 (3:50)	6.6	97
0.028	4.5	4/4 (4:15)	5.8	98
0.029	4.5	4/4 (7:00)	3.5	99
0.03	5.0	0/4	0	100

- a. Concentration of shellfish poison in test solution was calculated to be 273 mouse units/ml prior to the test.
- b. Number dead/number tested (average minutes:seconds to death).
- c. Concentration of shellfish poison in test solution after the test, mouse unit/ml.

In this particular series of tests, the shellfish poison was condensed to half its volume to obtain a concentration of 200 µg/ml. The condensation was accomplished by evaporation. A beaker containing 10 ml of the poison was placed in a ventilated cabinet; the contents were allowed to evaporate at room temperature to half the original volume. The blower was turned on to facilitate evaporation. The condensed shellfish poison was diluted with distilled water to the desired concentration. Mouse assay of the condensed poison indicated that the toxicity was not affected by the evaporation process.

b. Results

Results are shown in Table 4. These tests clearly demonstrated that a complete neutralization of the shellfish poison occurred under the above test conditions as long as the concentration of NaOCl was maintained at 3 ppm/µg of poison. Furthermore, the amount of NaOCl required to neutralize a given amount of poison is directly proportional to the concentration of the poison.

5. Experiment 5 with Sodium Hypochlorite

a. Test Procedure

To determine the effect of low temperature on the efficacy of the hypochlorite, another set of tests was conducted at 4 C. The procedure was similar to that previously described with the exception that the reaction between NaOCl and shellfish poison was carried out at 4 C. Both solutions were cooled to 4 C before mixing, and the contact times were 15 and 30 minutes. Two sets of tests were conducted with ratios of NaOCl to shellfish poison of 9:1 and 1:1 (v/v).

TABLE 4. INACTIVATION OF SHELLFISH POISON WITH NaOCl;^{a/} RATIO
OF NaOCl TO SHELLFISH POISON 1:1 (v/v);
CONTACT TIME, 15 MINUTES

NaOCl, %	Poison Concn, µg/ml	Approx. pH of Mixture	Mortality of Mice ^{b/}	Poison Concn ^{c/}	Poison Concn ^{d/}	Inactivation, %
0.006	20	4.0	0/4	54.6	0	100
0.012	40	4.0	0/4	109.2	0	100
0.018	60	4.5	0/4	163.8	0	100
0.024	80	4.5	0/4	218.4	0	100
0.03	100	5.0	0/4	273.0	0	100
0.036	120	5.0	0/4	327.6	0	100
0.042	140	5.0	0/4	382.2	0	100
0.048	160	5.0	0/4	438.8	0	100
0.054	180	5.0	0/4	491.4	0	100
0.06	200	5.0	0/4	546.0	0	100

- a. Concentrations of NaOCl and poison were based on the 3:1 ratio (3 ppm/µg).
b. Number dead/number tested (average minutes:seconds to death).
c. Initial concentration of shellfish poison in test solution, mouse unit/ml.
d. Final concentration of shellfish poison in test solution, mouse unit/ml.

b. Results

As summarized in Table 5, this experiment showed that a minimum of 4 ppm of NaOCl was required to neutralize 1 µg of poison at 4 C. Table 5 shows that, at 4 C, complete inactivation of the poison did not occur in a test solution containing 3 ppm of NaOCl/µg of poison, even though the contact time was extended to 30 minutes.

6. Experiment 6 with Sodium Hypochlorite

a. Test Procedure

A test procedure similar to that described earlier in this report was used to conduct another set of tests with NaOCl in the presence of sodium hydroxide (NaOH) solutions to determine the alkaline effect on the hypochlorite. The ratio of NaOCl to shellfish poison was 9:1 (v/v), and the contact times were 15 and 30 minutes. The alkaline solutions used in these tests were 0.01, 0.005, and 0.001% NaOH with pH values of 10.7, 10.4, and 9.7, respectively. The NaOCl was diluted with the above alkaline solutions to a desired concentration.

TABLE 5. INACTIVATION OF SHELLFISH POISON WITH NaOCl AT 4 C

Ratio of NaOCl to Poison, $\frac{a}{b}$ 9:1 (v/v)						Ratio of NaOCl to Poison, $\frac{b}{c}$ 1:1 (v/v)					
NaOCl, %	Approx. pH of Mixture	Contact Time, minutes	Mortality of Mice	Poison Concn/	Inact., %	NaOCl, %	Approx. pH of Mixture	Contact Time, minutes	Mortality of Mice	Poison Concn/	Inact., %
0.003	4.5	15	4/4 (4:04)	6.1	88	0.03	5.0	15	4/4 (3:45)	6.8	97
0.004	4.5	15	0/4	0	100	0.04	5.5	15	0/4	0	100
0.005	4.5	15	0/4	0	100	0.05	5.8	15	0/4	0	100
0.003	4.5	30	4/4 (4:00)	6.2	88	0.03	5.0	30	4/4 (4:00)	6.2	97
0.004	4.5	30	0/4	0	100	0.04	5.5	30	0/4	0	100
0.005	4.5	30	0/4	0	100	0.05	5.8	30	0/4	0	100
0.005 Control						0.03 Control					
Shellfish poison control (1:9 dilution)						Shellfish poison control (1:1 dilution)					
						4/4 (1:25)					
						4/4 (1:00)					

a. Concentration of shellfish poison in test solution was calculated to be 51.8 mouse units/ml prior to the test.

b. Concentration of shellfish poison in test solution was calculated to be 273 mouse units/ml prior to the test.

c. Number dead/number tested (average minutes:seconds to death).

d. Concentration of shellfish poison in test solution after the test, mouse unit/ml.

b. Results

Table 6 shows that Na H had a marked effect on the efficacy of the hypochlorite. A test solution containing 2 ppm of NaOCl was sufficient to neutralize 1 µg of poison when the solution contained 0.001% NaOH. When the NaOH was increased to 0.005%, a concentration of 1 ppm of NaOCl was sufficient to achieve complete neutralization.

c. CALCIUM HYPOCHLORITE

The $\text{Ca}(\text{OCl})_2$ used in these tests* contains 70% $\text{Ca}(\text{OCl})_2$ and 30% inert ingredients. The percentage of $\text{Ca}(\text{OCl})_2$ used in these tests was calculated on the 70% base. A stock solution of 0.7% was prepared and stored at 4 C. Chemical analysis of the $\text{Ca}(\text{OCl})_2$ at 0.7% indicated that it contained 0.6% available chlorine. Distilled water was used to dilute the chemical to a desired concentration. A freshly prepared solution was used for each test.

Solutions of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ at various concentrations were used as the $\text{Ca}(\text{OCl})_2$ neutralizer.

1. Test Procedure

In this series of tests, attempts were made to compare the efficacy of $\text{Ca}(\text{OCl})_2$ with that of NaOCl on the shellfish poison. For this reason the procedure used for the $\text{Ca}(\text{OCl})_2$ tests was similar to that of the NaOCl tests. Tests were conducted at 4 and 25 C and in the presence of alkaline solutions.

2. Results

The results summarized in Tables 7 to 9 showed that 1 µg of poison was destroyed by this chemical at concentrations of 3 ppm at room temperature (25 C) and 4 ppm at 4 C. With the presence of an alkali, a 1 µg of poison was neutralized by this chemical at concentrations of 2 ppm when the test solution contained 0.001% NaOH and of 1 ppm when the concentration of NaOH was 0.005%.

Tables 10 and 11 show that the efficacy of $\text{Ca}(\text{OCl})_2$ as a poison neutralizer is comparable to that of NaOCl in all respects.

d. IODINE, FORMALDEHYDE, BETA-PROPIOLACTONE, ROCCA, LYSOL, AND PERACETIC ACID

1. Test Procedure

Into a test tube that contained 5.4 ml of the disinfectant, 0.6 ml of the shellfish poison was added and mixed thoroughly. After 15, 30, and 60 minutes, 2 ml of the test solution were removed and acidified to pH 3.0 with the 1 N or 5 N HCl before injection into the mice.

* Produced by Omega Chemical Corp., New York 17, N.Y.

TABLE 6. INACTIVATION OF SHELLFISH POISON WITH NaOCl IN ALKALINE SOLUTION;
RATIO OF NaOCl TO SHELLFISH POISON, 9:1 (v/v)

0.002% NaOCl						0.001% NaOCl					
NaOH, %	Approx. pH of Mixture	Contact Time, minutes	Mortality of Mice ^a	Poison ^b / Concn ^c	Inact., %	NaOH, %	Approx. pH of Mixture	Contact Time, minutes	Mortality of Mice ^a	Poison ^b / Concn ^c	Inact., %
0.01	9.0	15	0/4	0	100	0.01	9.0	15	0/4	0	100
0.005	5.5	15	0/4	0	100	0.005	5.5	15	0/4	0	100
0.001	4.5	15	0/4	0	100	0.001	4.5	15	4/4 (1:44)	26	53
0.01	9.0	30	0/4	0	100	0.01	9.0	30	0/4	0	100
0.005	5.5	30	0/4	0	100	0.005	5.5	30	0/4	0	100
0.001	4.5	30	0/4	0	100	0.001	4.5	30	4/4 (2:15)	15.2	72
NaOCl 0.002% in 0.01% NaOH control						NaOCl 0.001% in 0.01% NaOH control					
Shellfish poison ^d / control 4/4 (1:23) (1:9 dilution)						Shellfish poison ^d / control 4/4 (1:24) (1:9 dilution)					

a. Mice (each) under tested (average minutes: seconds to death).

b. Concentration of shellfish poison in test solution after the test, mouse unit/ml.

c. Concentration of shellfish poison in test solution was calculated to be 58 mouse units/ml prior to the test.

d. Concentration of shellfish poison in test solution was calculated to be 55 mouse units/ml prior to the test.

TABLE 7. INACTIVATION OF SHELLFISH POISON WITH $\text{Ca}(\text{OCl})_2$;
RATIO OF $\text{Ca}(\text{OCl})_2$ TO SHELLFISH POISON, ^a/ 9:1 (v/v)

$\text{Ca}(\text{OCl})_2$, %	Approx. pH of Mixture	Contact Time, minutes	Temp. C	Mortality of Mice ^b	Poison Concn ^c /	Inactivation, %
0.004	4.5	15	25	0/4	0	100
0.003	4.5	15	25	0/4	0	100
0.002	4.5	15	25	4/4 (2:46)	10.6	84
0.004	4.5	30	25	0/4	0	100
0.003	4.5	30	25	0/4	0	100
0.002	4.5	30	25	4/4 (3:37)	7.2	89
0.004	4.5	15	4	0/4	0	100
0.003	4.5	15	4	4/4 (4:00)	6.2	90
0.004	4.5	30	4	0/4	0	100
0.003	4.5	30	4	4/4 (4:07)	6.1	91
0.004 Control			25	0/4		
Shellfish poison control (1:9 dilution)			25	4/4 (1:20)		

- a. Concentration of shellfish poison in test solution was calculated to be 66 mouse units/ml prior to the test.
b. Number dead/number tested (average minutes:seconds to death).
c. Concentration of shellfish poison in test solution after the test, mouse unit/ml.

TABLE 8. INACTIVATION OF SHELLFISH POISON WITH $\text{Ca}(\text{OCl})_2$;
RATIO OF $\text{Ca}(\text{OCl})_2$ TO SHELLFISH POISON, ^a/ 1:1 (v/v)

$\text{Ca}(\text{OCl})_2$, %	Approx. pH of Mixture	Contact Time, minutes	Temp. C	Mortality of Mice ^b	Poison Concn ^c /	Inactivation, %
0.04	4.5	15	25	0/4	0	100
0.03	4.5	15	25	0/4	0	100
0.02	4.5	15	25	4/4 (3:10)	8.6	97
0.04	4.5	30	25	0/4	0	100
0.03	4.5	30	25	0/4	0	100
0.02	4.5	30	25	4/4 (5:20)	4.5	98
0.04	4.5	15	4	0/4	0	100
0.03	4.5	15	4	0/4 (11 for 4 hr)	0	100
0.04 Control			25	0/4		
Shellfish poison control (1:1 dilution)			25	4/4 (1:00)		

- a. Concentration of shellfish poison in test solution was calculated to be 273 mouse units/ml prior to the test.
b. Number dead/number tested (average minutes:seconds to death).
c. Concentration of shellfish poison in test solution after the test, mouse unit/ml.

TABLE 9. INACTIVATION OF SHELLFISH POISON WITH $\text{Ca}(\text{OCl})_2$ IN ALKALINE SOLUTIONS;
RATIO OF SHELLFISH POISON TO $\text{Ca}(\text{OCl})_2$, 9:1 (v/v)

0.002% $\text{Ca}(\text{OCl})_2$						0.001% $\text{Ca}(\text{OCl})_2$					
NaOH, %	Approx. pH of Mixture	Contact Time, minutes	Mortality of Mice ^a	Poison ^b / Concn ^b	Inact., %	NaOH, %	Approx. pH of Mixture	Contact Time, minutes	Mortality of Mice ^a	Poison ^b / Concn ^b	Inact., %
0.01	9.0	15	0/4	0	100	0.01	9.0	15	0/4	0	100
0.005	5.5	15	0/4	0	100	0.005	5.5	15	0/4	0	100
0.001	4.5	15	0/4	0	100	0.001	4.5	15	4/4 (1:50)	24.4	58
0.01	9.0	30	0/4	0	100	0.01	9.0	30	0/4	0	100
0.005	5.5	30	0/4	0	100	0.005	5.5	30	0/4	0	100
0.001	4.5	30	0/4	0	100	0.001	4.5	30	4/4 (1:56)	21	65
$\text{Ca}(\text{OCl})_2$ 0.002% in 0.01% NaOH control						$\text{Ca}(\text{OCl})_2$ 0.001% in 0.01% NaOH control					
Shellfish poison ^d / control 4/4 (1:20) (1:9 dilution)						Shellfish poison ^d / control 4/4 (1:22) (1:9 dilution)					

a. Number dead/number tested (average minutes:seconds to death).

b. Concentration of shellfish poison in test solution after the test, mouse unit/ml.

c. Concentration of shellfish poison in test solution was calculated to be 66 mouse units/ml prior to the test.

d. Concentration of shellfish poison in test solution was calculated to be 60 mouse units/ml prior to the test.

TABLE 10. COMPARISON OF EFFICACY OF NaOCl AND Ca(OCl)₂ ON SHELLFISH POISON;
RATIO OF DISINFECTANTS TO SHELLFISH POISON, 9:1 (v/v);
CONTACT TIME, 15 MINUTES

NaOCl					Ca(OCl) ₂						
NaOCl, %	Temp, C	Mortality of Mice ^a	Poison Concn ^b / Concn ^c	Poison Concn ^d / Concn ^e	Inact., %	Ca(OCl) ₂ , %	Temp, C	Mortality of Mice ^a	Poison Concn ^b / Concn ^c	Poison Concn ^d / Concn ^e	Inact., %
0.003	25	0/4	66.0	0	100	0.003	25	0/4	66	0	100
0.002	25	4/4 (3:40)	66.0	7.0	89	0.002	25	4/4 (2:46)	66	10.6	84
0.004	4	0/4	51.8	0	100	0.004	4	0/4	66	0	100
0.003	4	4/4 (4:04)	51.8	6.1	88	0.003	4	4/4 (4:00)	66	6.2	90
0.002 ^d	25	0/4	58.0	0	100	0.002 ^d	25	0/4	66	0	100
0.001 ^e	25	0/4	55.0	0	100	0.001 ^e	25	0/4	60	0	100
0.001 ^d	25	4/4 (1:44)	55.0	26.0	53	0.001 ^d	25	4/4 (1:56)	60	21	65

a. Number of deaths/number tested (average minutes:seconds to death).

b. Concentration of shellfish poison in test solution before the test, mouse unit/ml.

c. Concentration of shellfish poison in test solution after the test, mouse unit/ml.

d. The disinfectants were prepared in 0.001% NaOH.

e. The disinfectants were prepared in 0.005% NaOH.

TABLE 11. COMPARISON OF EFFICACY OF NaOCl AND Ca(OCl)₂ ON SHELLFISH POISON;
RATIO OF DISINFECTANTS TO SHELLFISH POISON, 1:1 (v/v);
CONTACT TIME, 15 MINUTES

NaOCl					Ca(OCl) ₂				
NaOCl, %	Temp, C	Mortality of Mice ^a / Concn ^b	Poison Concn ^c / Concn ^b	Inact., %	Ca(OCl) ₂ , %	Temp, C	Mortality of Mice ^a / Concn ^c	Poison Concn ^c / Concn ^b	Inact., %
0.03	25	0/4	273	0	0.03	25	0/4	273	0
0.02	25	4/4 (2:04)	273	17.6	0.02	25	4/4 (3:10)	273	8.6
0.04	4	0/4	273	0	0.04	4	0/4	273	0
0.03	4	4/4 (3:45)	273	6.8	0.03	4	0/4 (11 for 4 hr)	273	0

a. Number of deaths/number tested (average minutes:seconds to death).

b. Concentration of shellfish poison in test solution before the test, mouse unit/ml.

c. Concentration of shellfish poison in test solution after the test, mouse unit/ml.

In the I_2 test, 0.2 ml of the 10% $Na_2S_2O_3 \cdot 5H_2O$ was used as the I_2 neutralizer. In the Roccal test, the test solution was neutralized with 0.2 ml of a 10% Tamol N solution (Tamol N is a neutral salt of a condensed aryl sulfonic acid). In the peracetic acid test, 2 drops of $KMnO_4$ (3%) and 0.2 ml of the 50% $Na_2S_2O_3 \cdot 5H_2O$ were added to the test solution before adjusting it to pH 3.0 and inoculating it into mice.

Three sets of controls were used in these tests. One group of mice was inoculated with 0.4 ml of the disinfectant to serve as the disinfectant control; another group of mice was inoculated with 0.4 ml of the 1:9 dilution of the poison to use as the poison control. The third group of mice was inoculated with the 1:9 dilution of the poison that was similarly treated with the disinfectant neutralizer. The results of these tests are shown in Table 12.

2. Results

Data from the controls indicated that 47% of the toxicity of the poison was destroyed by the I_2 neutralizer; 82% of the toxicity was destroyed by the Roccal neutralizer; 85% of the toxicity was destroyed by the peracetic acid neutralizers. This loss of toxicity was probably due to the salt effect of the Tamol N and the thiosulfate solutions. When the poison was treated with the peracetic acid neutralizers, the oxidizing action of the $KMnO_4$ might have caused most of the poison destruction. For this reason, a test with $KMnO_4$ was conducted.

E. POTASSIUM PERMANGANATE

1. Test Procedure

Two-tenths milliliter of the shellfish poison was added to a test tube that contained 1.8 ml of the 0.1% $KMnO_4$ solution. After 15 minutes of contact time, the test solution was acidified with 5 N HCl to pH 3.0, and 0.4 ml of the solution was inoculated into the mice. The same procedure was repeated with the 0.05 and 0.01% $KMnO_4$ solutions.

2. Results

From Table 13 it is apparent that the poison was completely neutralized with the 0.1% $KMnO_4$ solution. The fact that all the mice injected with the test solution containing 0.05% $KMnO_4$ did not die but were ill for 4 hours indicated that the end point for complete inactivation lies between the 0.05 and the 0.1% $KMnO_4$ concentrations. Further study on the inactivation of shellfish poison with $KMnO_4$ was not possible because of the lack of shellfish poison.

TABLE 12. EFFECT OF I₂, HCHO, BPL, ROCCAL, LYSOL, AND PERACETIC ACID ON SHELLFISH POISON^{a/}

Disinfectant	Concn, %	Approx. pH of Mixture	Contact Time, minutes	Mortality of Mice ^{b/}	Poison Concn ^{c/}	Inactivation, %
I ₂	0.5	3.5	15	4/4 (1:55)	21	68
I ₂	0.5	3.5	30	4/4 (1:40)	30	54
I ₂	0.5	3.5	60	4/4 (1:42)	30	54
I ₂ control	0.5			0/4		
HCHO	0.5	4.0	15	4/4 (1:23)	60	9
HCHO	0.5	4.0	30	4/4 (1:20)	66	0
HCHO	0.5	4.0	60	4/4 (1:23)	60	9
HCHO control	0.5			0/4		
BPL	1.0	3.5	15	4/4 (1:44)	26	60
BPL	1.0	3.5	30	4/4 (1:24)	55	16
BPL	1.0	3.5	60	4/4 (1:26)	51	22
BPL control	1.0			0/4		
Roccal	1.0	3.0	15	4/4 (1:31)	41	38
Roccal	1.0	3.0	30	4/4 (1:35)	35	47
Roccal	1.0	3.0	60	4/4 (1:47)	46	30
Roccal control	1.0			0/4		
Lysol	5.0	8.0	15	4/4 (1:30)	41	38
Lysol	5.0	8.0	30	4/4 (1:20)	66	
Lysol	5.0	8.0	60	4/4 (1:24)	55	16
Lysol control	5.0			0/4		
Peracetic acid	1.0	3.0	15	4/4 (1:55)	21	68
Peracetic acid	1.0	3.0	30	4/4 (2:24)	13	80
Peracetic acid	1.0	3.0	60	4/4 (3:20)	8	88
Peracetic acid control	1.0			0/4		
Poison plus I ₂ neutralizer				4/4 (1:35)	35	47
Poison plus Roccal neutralizer				4/4 (2:35)	12	82
Poison plus peracetic acid neutralizer				4/4 (2:50)	10	85
Shellfish poison control (1:9 dilution)				4/4 (1:20)		

a. Concentration of shellfish poison in test solution was calculated to be 66 mouse units/ml prior to the test.

b. Number dead/number tested (average minutes:seconds to death).

c. Concentration of shellfish poison in test solution after the test, mouse unit/ml.

TABLE 13. INACTIVATION OF SHELLFISH POISON^a WITH KMnO_4 ; RATIO OF KMnO_4 TO SHELLFISH POISON 9:1; CONTACT TIME, 15 MINUTES

KMnO_4 , %	Approx. pH of Mixture	Mortality of Mice ^b	Poison Concn ^c /	Inactivation, %
0.1	5.0	0/4	0	100
0.05	4.5	0/4 (111 for 4 hr)	0	100
0.01	4.0	4/4 (1:41)	30	46
0.1 Control		0/4		
Shellfish poison control (1:9 dilution)		4/4 (1:20)		

- Concentration of shellfish poison in test solution was calculated to be 66 mouse units/ml prior to the test.
- Number of deaths/number tested (average minutes:seconds to death).
- Concentration of shellfish poison in test solution after the test, mouse unit/ml.

F. ETHYLENE OXIDE

1. Test Procedure

Two 30-ml beakers, one containing 0.5 ml of shellfish poison and the other 0.5 ml of distilled water, were placed into a carboxyclave. The distilled water served as the ethylene oxide control. A vacuum was drawn until the pressure in the carboxyclave was down to -17 pounds. Then a can of ethylene oxide mixture (600 g) containing 12% ethylene oxide, 44% dichlorodifluoromethane, and 44% trichlorofluoromethane was introduced into the carboxyclave. The concentration of ethylene oxide inside the chamber was approximately 12 g/ft³. The shellfish poison and the distilled water were exposed to these gases for 16 hours. At the end of the exposure period, the beakers were removed from the carboxyclave and exposed to the air for 24 hours to allow evaporation of ethylene oxide from the beakers. Because all the liquids in the beakers were evaporated, 5 ml distilled water were added to each beaker to reconstitute the solution. The solutions were shaken thoroughly and adjusted to pH 3.0 with 1 N HCl before inoculating into mice. Four mice inoculated with 0.4 ml of the 1:9 dilution of the shellfish poison were used as the shellfish poison controls.

2. Results

None of the ethylene oxide control mice died. All mice inoculated with the shellfish poison that was exposed to the ethylene oxide died in 1 minute 29 seconds, indicating that 38% of the poison was inactivated by this chemical. All the shellfish poison control mice died in 1 minute 20 seconds.

G. COMPARATIVE EFFECT OF TEST CHEMICALS

The comparative effect of all the test chemicals on shellfish poison is given in Table 14. The chemicals are listed in descending order of effectiveness.

Table 14 shows that, of the chemicals tested, calcium hypochlorite, sodium hypochlorite, and potassium permanganate were the most effective in neutralizing the toxicity of the poison. Both calcium hypochlorite and sodium hypochlorite were 100% effective at concentrations of 0.003%. Potassium permanganate was effective at 0.05%. Sodium hydroxide was effective at 0.3% but required 24 hours to produce a 53% inactivation. Ethylene oxide at a concentration of 12 g/ft³ was 38% effective after 16 hours. After 1 hour of exposure, beta-propiolactone was 22% effective at 1%. Lysol was 16% effective at 5%. Formaldehyde was 9% effective at 0.5%. Iodine was 7% effective at 0.5%. Peracetic acid was 5% effective at 1%. Roccal at a 1% concentration had no effect on the poison.

TABLE 14. COMPARATIVE EFFECT OF TEST CHEMICALS ON SHELLFISH POISON;
RATIO OF CHEMICAL TO POISON, 9:1 (v/v)

Chemical	Concn of Chemical, %	Concn of Poison, mouse unit/ml	Contact Time, minutes	Inact., %
Calcium hypochlorite	0.003	66	15	100
Sodium hypochlorite	0.003	66	15	100
Potassium permanganate	0.05	66	15	100
Sodium hydroxide	0.3	5.9	1,440	53
Ethylene oxide	12.0 (g/ft ³)	66	960	38
Beta-propiolactone	1.0	66	60	22
Lysol	5.0	66	60	16
Formaldehyde	0.5	66	60	9
Iodine	0.5	66	60	7
Peracetic acid	1.0	66	30	5
Roccal	1.0	66	60	0

IV. DISCUSSION

The chemistry of shellfish poison has been studied extensively by Schantz, Mold, McFarren, and their co-workers. Studies by Schantz et al.⁷ have shown that the poison can be destroyed either by oxidation or reduction. By using hydrogen gas as a reducing agent in the presence of a platinum catalyst (Mold et al.), and $\text{Ba}(\text{OH})_2$ exposed to the oxygen of the air (Wintersteiner et al.), other investigators demonstrated that the destruction of shellfish poison was directly proportional to the H_2 or O_2 uptake.

Because the poison is susceptible to oxidation, it is not too surprising to find that it is inactivated with NaOCl , $\text{Ca}(\text{OCl})_2$, or KMnO_4 because all these chemicals are strong oxidizing agents. Through the process of oxidation, the poison molecule is broken up into fragments and becomes nontoxic. Products such as guanidopropionic acid, urea, ammonia, carbon dioxide, and guanidine were isolated and identified by Schantz et al.⁷ when the poison was treated with periodate and permanganate or strong acid hydrolysis.

It is interesting to note that $\text{Ca}(\text{OCl})_2$, even though it contains 15% less free available chlorine than NaOCl at any given concentration, is just as effective against the shellfish poison as NaOCl under any of the above test conditions. This fact suggests that the amount of free available chlorine present in the hypochlorite has little or no effect on the shellfish poison, but rather it is the amount of available oxygen in the hypochlorite that plays a vital role in the destruction of the shellfish poison. Brazis and co-workers³ also found that free available chlorine at 2 mg per liter had little or no effect on the toxicity of the shellfish poison when 4.7 mg of shellfish poison were exposed to this concentration of free available chlorine at pH 7.0 and 25 C for 2 hours.

Both sodium hypochlorite and calcium hypochlorite are effective as shellfish poison neutralizers at 25 and 4 C. Jones, Hoffman, and Phillips¹⁰ have also shown that NaOCl remains very effective as a disinfectant against *Bacillus subtilis* var. *niger* spores, even at subzero temperatures. At 25 C, a minimum concentration of 3 ppm of sodium hypochlorite or calcium hypochlorite is required to neutralize 1 μg of shellfish poison in 15 minutes. Furthermore, the amount of hypochlorite required to neutralize a given amount of shellfish poison is directly proportional to the concentration of the poison (Table 4). Instantaneous inactivation could occur if a higher concentration of hypochlorite were used.

At 4 C, the amount of hypochlorite required to inactivate 1 μg of shellfish poison appears to be 4 ppm.

The presence of alkali in the hypochlorite solution tends to enhance the effectiveness of this disinfectant. A concentration of 1 ppm is sufficient to neutralize 1 μg of shellfish poison if the hypochlorite is prepared in 0.005% NaOH solution or 2 ppm if it is prepared in 0.001% NaOH .

KMnO_4 is not as effective as hypochlorite. It requires approximately 50 ppm to neutralize 1 μg of shellfish poison.

Both sodium hypochlorite and calcium hypochlorite are approximately 17 times as effective as potassium permanganate.

Other disinfectants such as sodium hydroxide, ethylene oxide, beta-propiolactone, Lysol, formaldehyde, iodine, peracetic acid, and Roccal are not as effective as the hypochlorites and the potassium permanganate.

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<p>The neutralization of shellfish poison by 11 chemical disinfectants was studied. Both sodium hypochlorite and calcium hypochlorite were effective in neutralizing the toxicity of the poison at 3 ppm/μg of poison at room temperature (25 C) and at 4 ppm/μg of poison at 4 C. Alkalinity in the hypochlorite solutions tends to enhance the effectiveness of these disinfectants.</p> <p>Potassium permanganate was effective as a poison neutralizer at 50 ppm/μg of poison at 25 C.</p> <p>Sodium hydroxide, ethylene oxide, beta-propiolactone, Lysol, formaldehyde, iodine, peracetic acid, and Roccal were not as effective as the hypochlorites and the permanganate.</p>														
14. Key Words														
<table border="0"> <tr> <td>*Shellfish poison</td> <td>Poisons</td> </tr> <tr> <td>*Neutralizers</td> <td>Disinfectants</td> </tr> <tr> <td>Neutralizing</td> <td>Disinfection</td> </tr> <tr> <td>Calcium hypochlorites</td> <td>Potassium permanganate</td> </tr> <tr> <td>Hypochlorites</td> <td></td> </tr> <tr> <td>Sodium hypochlorite</td> <td></td> </tr> </table>			*Shellfish poison	Poisons	*Neutralizers	Disinfectants	Neutralizing	Disinfection	Calcium hypochlorites	Potassium permanganate	Hypochlorites		Sodium hypochlorite	
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